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Azaelectrocyclization on cell surface: convenient and general approach to chemical biology research

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ABSTRACT

Convenient and general method for live cell surface labeling was established. Azide-containing fluorescences were initially conjugated with the unsaturated ester aldehyde containing the dibenzocyclooctyne (DIBO), by the strain-promoted click reaction. The resulting probes were then treated with the live cells in one-pot process, efficiently labeling the surface lysines by azaelectrocyclization under the mild conditions.

1. Introduction

Covalent chemical labeling of live cells has garnered significant attention in the fields related to molecular imaging,[1] and has broad and general applicability to both in vitro and in vivo studies of live cells.[2] Bioorthogonal approaches, which can be combined with biological techniques, have actively been investigated.[3,4] Successful examples include Bertozzi's Staudinger ligation[5,6] and strain-accelerated Huisgen 1,3-cycloaddtion reaction.[7–10] These methods have been applied to labeling of cells expressing oligosaccharides with azido-containing sugar residues on the cell surfaces through biosynthetic pathways,[8] which subsequently reacted with the methoxycarbonyl phenyldiphenylphosphine or cyclooctyne derivatives. Variety of the reactive cyclooctyne derivatives have continuously been developed in pursuit of the efficient bioconjugation on the live cell surfaces by this strategy.[10]

On the other hand, we recently developed an amine-based labeling through rapid 6π-azaelectrocyclization.[11–13] We have used this method to efficiently and conveniently introduce the fluorescent groups to the amino groups on the cell surfaces[14,15] via a reaction with unsaturated aldehyde probes (such as probe **1a** in Scheme 1a) at very low concentrations (can be decreased down to 10^{-8} M) within a short time (10 min) at room temperature. Although our reaction is non-bioorthogonal to the primary amino groups on cell surfaces, under such mild reaction conditions the most exposed and densely expressed amines i.e., lysines of membrane proteins and/or ethanol amine derivatives at cell surfaces react rapidly and selectively; hence minimizing the indiscriminative amino modification as well as the significant probe internalization, which cause the interferences with their native functions. Based on the method, in vivo imaging of the lymphocytes labeled by the near-infrared dye was also achieved as an application of the present protocol; the trafficking of the cells into the immune-related organs was clearly visualized with markedly high imaging contrasts.[14]

In widely applying our electrocyclization chemistry to the cell surface labeling by various fluorescence dyes and/or the other small molecules,[15a] however, a 'generality problem', has still remained unsolved. Namely, the unsaturated aldehydes **1a** are to be synthesized for each different fluorescence probe. Although **1a** with TAMRA or Cy5 dyes could readily be prepared, some fluorescence structures, such as fluoresceins, were also found to decompose during the oxidation process from the precursor alcohol to the probes **1a**. In order to generalize the cell-surface labeling, we have then report a two-step protocol by combining our electrocyclization chemistry with the biotin/avidin and/or biotin/*anti*-biotin monoclonal antibody (mAb) interactions, as shown in Scheme 1b.[15b] Thus, the cell surfaces are initially biotinylated by azaelectrocyclization using the probe **1b**, and then treated with the fluorescence-labeled avidin or the *anti*-biotin antibody.

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